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 NEWS  1          Web Page for STN Seminar Schedule - N. America
 NEWS  2  AUG 15  CAOLD to be discontinued on December 31, 2008
 NEWS  3  OCT 07  EPFULL enhanced with full implementation of EPC2000
 NEWS  4  OCT 07  Multiple databases enhanced for more flexible patent
                  number searching
 NEWS  5  OCT 22  Current-awareness alert (SDI) setup and editing
                  enhanced
 NEWS  6  OCT 22  WPIDS, WPINDEX, and WPIX enhanced with Canadian PCT
                  Applications
 NEWS  7  OCT 24  CHEMLIST enhanced with intermediate list of
                  pre-registered REACH substances
 NEWS  8  NOV 21  CAS patent coverage to include exemplified prophetic
                  substances identified in English-, French-, German-,
                  and Japanese-language basic patents from 2004-present
 NEWS  9  NOV 26  MARPAT enhanced with FSORT command
 NEWS 10  NOV 26  MEDLINE year-end processing temporarily halts
                  availability of new fully-indexed citations
 NEWS 11  NOV 26  CHEMSAFE now available on STN Easy
 NEWS 12  NOV 26  Two new SET commands increase convenience of STN
                  searching
 NEWS 13  DEC 01  ChemPort single article sales feature unavailable
 NEWS 14  DEC 12  GBFULL now offers single source for full-text
                  coverage of complete UK patent families
 NEWS 15  DEC 17  Fifty-one pharmaceutical ingredients added to PS

 NEWS EXPRESS JUNE 27 08 CURRENT WINDOWS VERSION IS V8.3,
              AND CURRENT DISCOVER FILE IS DATED 23 JUNE 2008.

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=> S G-CSF (P)(inclusion Body) AND pd<=20030611
   1 FILES SEARCHED...
L1          27 G-CSF (P)(INCLUSION BODY) AND PD<=20030611

=> Dup Rem L1
PROCESSING COMPLETED FOR L1
L2          18 DUP REM L1 (9 DUPLICATES REMOVED)
               ANSWERS '1-4' FROM FILE MEDLINE
               ANSWERS '5-18' FROM FILE CAPLUS

=> S L2 AND Non-denatur?
L3           1 L2 AND NON-DENATUR?

=> D ibib abs l3

L3   ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER:        2002:127621  CAPLUS
DOCUMENT NUMBER:         137:322125
TITLE:                   Immobilized metal ion affinity refolding of
                         recombinant proteins
AUTHOR(S):               Rozenaite, V.; Baskeviciute, B.; Luksa, V.; Bumelis,
                         V.; Pesliakas, H.
CORPORATE SOURCE:        Institute of Biotechnology, Vilnius, LT-2028,
                         Lithuania
SOURCE:                  Biologija (2001), (4), 25-29
                         CODEN: BOLOE8; ISSN: 1392-0146
PUBLISHER:               Lietuvos Mokslu Akademijos Leidykla
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   Renaturation of recombinant proteins expressed in E. coli and accumulated
     as inclusion bodies by immobilized metal ion affinity
     chromatog. (IMAC) technique was evaluated.  Recombinant human
     interleukin-3 (IL-3), granulocyte-colony stimulating factor (G-
     CSF) and granulocyte macrophage-colony stimulating factor
     (GM-CSF), all possess metal-chelating sites in their sequence and were
     used for investigation of their renaturation upon denatured macromol.
     interaction with meta lions charged Sepharose iminodiacetate (IDA) gels.
     The efficiency of correctly folded protein generation was studied
     depending on the concentration of guanidine-HCl in a loading buffer of
     inclusion bodies solution, type of metal ion, pH and
     protein loading.  The IMAC procedure was shown to be promising and enabled
     to recover part of the target protein in non-denaturing
     conditions with the protein-dependent yield.
REFERENCE COUNT:         20    THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS
                               RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT
```

=> D Ti L2 1-18

L2 ANSWER 1 OF 18 MEDLINE on STN DUPLICATE 1
TI Expression in Escherichia coli and purification of the functional feline granulocyte colony-stimulating factor.

L2 ANSWER 2 OF 18 MEDLINE on STN DUPLICATE 2
TI Expression, purification, and in vitro biological activities of recombinant bovine granulocyte-colony stimulating factor.

L2 ANSWER 3 OF 18 MEDLINE on STN DUPLICATE 3
TI Chediak-Higashi-Steinbrinck syndrome (CHS) in a 27-year-old woman--effects of G-CSF treatment.

L2 ANSWER 4 OF 18 MEDLINE on STN DUPLICATE 4
TI Evaluation of penicilloyl proteins of allergic impurity in gene engineering drugs.

L2 ANSWER 5 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Mutation of surface-exposed histidine residues of recombinant human granulocyte-colony stimulating factor (Cys17Ser) impacts on interaction with chelated metal ions and refolding in aqueous two-phase systems

L2 ANSWER 6 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Two-step chromatography - a unique procedure for purification of granulocyte colony stimulating factor (G-CSF) from recombinant E.coli

L2 ANSWER 7 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Plasmid vectors and recombinant production of human granulocyte colony stimulating factor (G-CSF) in Escherichia coli

L2 ANSWER 8 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Immobilized metal ion affinity refolding of recombinant proteins

L2 ANSWER 9 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Cloning, expression, purification and function research of recombinant human FLT3 ligand

L2 ANSWER 10 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Extraction and renaturation of recombinant human granulocyte colony stimulating factor inclusion body

L2 ANSWER 11 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Process for the purification of recombinant human granulocyte-colony stimulating factor in the form of inclusion body from yeast

L2 ANSWER 12 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI An artificial operon for chaperonin synthesis and its use in preventing formation of inclusion bodies in protein manufacture in bacterial hosts

L2 ANSWER 13 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Preparation of fusion protein containing Escherichia coli thioredoxin derivative using a soluble expression system

L2 ANSWER 14 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Over-expression of G-CSF in Escherichia coli and fast purification protocol

L2 ANSWER 15 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Expression of cDNA for recombinant human granulocyte colony-stimulating

factor in Escherichia coli and characterization of the protein

L2 ANSWER 16 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Recombinant expression and identification of human granulocyte colony-stimulating factor cDNA in Escherichia coli

L2 ANSWER 17 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Improving the resolubilization of proteins synthesized in an heterologous host and accumulated as inclusion bodies

L2 ANSWER 18 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
TI Use of IgA proteinase to manufacture recombinant proteins without terminal methionine with bacteria

=> D ibib abs L2 1,2,9-17

L2 ANSWER 1 OF 18 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2002698498 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12459164
TITLE: Expression in Escherichia coli and purification of the functional feline granulocyte colony-stimulating factor.
AUTHOR: Yamamoto Akira; Iwata Akira; Saitoh Toshiki; Tuchiya Kotaro; Kanai Tomoko; Tsujimoto Hajime; Hasegawa Atsuhiko; Ishihama Akira; Ueda Susumu
CORPORATE SOURCE: Nippon Institute for Biological Science, Shin-Machi 9-2221-1, Ome, Tokyo 198-0024, Japan.. yamagen@nibs.or.jp
SOURCE: Veterinary immunology and immunopathology, (2002 Dec) Vol. 90, No. 3-4, pp. 169-77.
Journal code: 8002006. ISSN: 0165-2427.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200310
ENTRY DATE: Entered STN: 17 Dec 2002
Last Updated on STN: 31 Oct 2003
Entered Medline: 30 Oct 2003
AB Feline granulocyte colony-stimulating factor (G-CSF) with an N-terminal histidine hexamer tag was expressed as inclusion bodies in E. coli. The G-CSF solubilized in 6 M guanidine solution was absorbed onto a Ni-NTA column and, after washing with decreasing concentrations of guanidine, eluted with imidazole in a soluble and apparently pure form. The activity of the recombinant feline G-CSF was 3 x 10(6)U/mg protein, as assayed by its stimulatory effect on NFS-60 cell proliferation. When a low level of purified feline G-CSF was administered once a day for two successive days to cats, the number of neutrophil increased 4-fold while the levels of other blood cell types remained virtually unchanged. Daily administration of G-CSF for a total of 11 days led to a more than 10-fold increase in neutrophils, an 8-fold increase in the number of monocytes and 2-fold increase in lymphocytes. No severe side effects or antibody production was observed in cats after administration of G-CSF.

L2 ANSWER 2 OF 18 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2001452938 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11498246
TITLE: Expression, purification, and in vitro biological

activities of recombinant bovine granulocyte-colony stimulating factor.
AUTHOR: Heidari M; Harp J A; Kehrli M E Jr
CORPORATE SOURCE: Periparturient Diseases of Cattle Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA 50010, USA.. mheidari@nadc.ars.usda.gov
SOURCE: Veterinary immunology and immunopathology, (2001 Aug 30) Vol. 81, No. 1-2, pp. 45-57.
Journal code: 8002006. ISSN: 0165-2427.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200109
ENTRY DATE: Entered STN: 13 Aug 2001
Last Updated on STN: 17 Sep 2001
Entered Medline: 13 Sep 2001

AB Neutrophils are essential components of the innate immune system and they play a critical role in the defense of host against bacterial and fungal infections. The colony stimulating factors are a class of glycoproteins that are required for proliferation, differentiation, and functional activation of hematopoietic progenitor cells. Granulocyte-colony stimulating factor (G-CSF) is a member of this regulatory family of cytokines that specifically stimulates proliferation and maturation of precursor cells in the bone marrow into fully differentiated and functional neutrophils. G-CSF also modulates the biological activities of mature neutrophils in circulation. A bovine G-CSF (bG-CSF) cDNA clone (previously isolated and sequenced in our laboratory) was expressed in Escherichia coli and the biological activities of the solubilized protein from purified inclusion bodies were examined. Flow cytometric analysis of membrane antigen density of neutrophils activated with bG-CSF revealed an upregulation in the expression of CD11a (>114%), CD11b (>148%), CD11c (>87%), and CD18 (>109%). Expression of L-selectin was decreased by more than 43%. There was no change, however, in the expression of CD14. These findings indicate that recombinant bG-CSF (rbG-CSF) expressed in E. coli is biologically active and exerts the same type of effects on neutrophils in vitro as those of human G-CSF (hG-CSF).

L2 ANSWER 9 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2001:27563 CAPLUS
DOCUMENT NUMBER: 135:221992
TITLE: Cloning, expression, purification and function research of recombinant human FLT3 ligand
AUTHOR(S): Zhang, Wei-jie; Liu, Jing-zhong; Lu, Xing; Pei, Xue-tao; Sun, Zhi-xian
CORPORATE SOURCE: Beijing Chaoyang Hospital, Beijing, 100020, Peop. Rep. China
SOURCE: Shengwu Gongcheng Xuebao (2000), 16(6), 708-712
CODEN: SGXUED; ISSN: 1000-3061
PUBLISHER: Kexue Chubanshe
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB Clone human FLT3 ligand gene, stablish a highly efficient expression system of rhFL in E. coli and a suitable purification method of expression products. A cDNA encoding soluble FL was cloned through RT-nested PCR from the total RNA extracted from human peripheral blood mononuclear cells and identified by analyzing the nucleotide sequences, then introduced into pProEXHT plasmid to express a 6 x His-FL fusion protein in E. coli. The fusion protein expressed in inclusion body was

isolated, solubilized and refolded, and then purified by chromatog. on a Ni-chelating affinity column. Its activity was detected by stimulating CD34+ cells to expanse. RhFL gene with a length of 481 bp was isolated. The expression amount of rhFL reached to 15% of total bacterial proteins and the purity of rhFL was 90% after MCAC. RhFL + G-CSF + EPO stimulated CD34+ cells to expanse up to 400 times. The purified rhFL had a powerful activity to stimulate hematopoietic stem cells to expanse in vitro.

L2 ANSWER 10 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2000:35378 CAPLUS
DOCUMENT NUMBER: 132:189772
TITLE: Extraction and renaturation of recombinant human granulocyte colony stimulating factor inclusion body
AUTHOR(S): Ma, Li; Ning, Yunshan; Fang, Xiangdong; Linlai, Xinmei; Yu, Lin; Wang, Xiaoning
CORPORATE SOURCE: Institute of Molecular Immunology, First Medical University of PLA, Canton, 510515, Peop. Rep. China
SOURCE: Zhongguo Shenghua Yaowu Zazhi (1999), 20(5), 221-223
CODEN: ZSYZFP; ISSN: 1005-1678
PUBLISHER: Zhongguo Shenghua Yaowu Zazhi Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB Expts. were carried out to study the refolding of inclusion body of recombinant human granulocyte colony stimulating factor (rhG-CSF). The design involved using a new method for purification of inclusion body by a novel invention. Recovery of refolded G-CSF is more than 90% and the specific bioactivity of rhG-CSF is more than 80% of native form. By using a simple, novel inclusion body purification method, high efficiency of refolding of rhG-CSF is attained.

L2 ANSWER 11 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2004:844614 CAPLUS
DOCUMENT NUMBER: 142:43731
TITLE: Process for the purification of recombinant human granulocyte-colony stimulating factor in the form of inclusion body from yeast
INVENTOR(S): Lee, Snag Mi; Kim, Se Hoon; Kim, Kyu Wan; Kim, Kyu Don
PATENT ASSIGNEE(S): Lg Chemical Co., Ltd, S. Korea
SOURCE: Repub. Korea, No pp. given
CODEN: KRXXFC
DOCUMENT TYPE: Patent
LANGUAGE: Korean
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
KR 160934	B1	19981116	KR 1996-649	19960115 <--

PRIORITY APPLN. INFO.: KR 1996-649 19960115
AB A method for purifying recombinant human granulocyte colony stimulating factors(rhG-CSFs) useful for treating immunity-related diseases and leucosis, etc. is provided. Recombinant human granulocyte colony stimulating factors(rhG-CSFs) consist of 174 amino acids, and contain two disulfide bonds and a glycosylation site. The method for purifying rhG-CSFs comprises the steps of: incubating yeasts expressing rhG-CSFs(pYLBC A/G-UB-Met G-CSF/DC04); obtaining inclusion bodies containing rhG-CSFs from yeasts by disruption with glass beads; dissolving inclusion bodies in 6M guanidine solution; forming disulfide-bonds by oxidation of

inclusion bodies; precipitating rhG-CSFs and re-dissolving them in 30 mM sodium acetic acid solution; and purifying rhG-CSF by passing a solution through a cation-exchange chromatog., a hydrophobic chromatog., and a gel-filtration chromatog., sequentially.

L2 ANSWER 12 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1999:12471 CAPLUS
DOCUMENT NUMBER: 130:62032
TITLE: An artificial operon for chaperonin synthesis and its use in preventing formation of inclusion bodies in protein manufacture in bacterial hosts
INVENTOR(S): Sogo, Kazuyo; Yanagi, Hideki; Yura, Takashi
PATENT ASSIGNEE(S): Hsp Research Institute, Inc., Japan
SOURCE: Eur. Pat. Appl., 22 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 885967	A2	19981223	EP 1998-111348	19980619 <--
EP 885967	A3	20000621		
EP 885967	B1	20040616		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 11009274	A	19990119	JP 1997-180558	19970620 <--
JP 3344618	B2	20021111		
CA 2235468	A1	19981220	CA 1998-2235468	19980619 <--
CA 2235468	C	20080617		
US 6159708	A	20001212	US 1998-100110	19980619 <--
AT 269410	T	20040715	AT 1998-111348	19980619

PRIORITY APPLN. INFO.: JP 1997-180558 A 19970620
AB An artificial operon containing the genes for the chaperones DnaK, DnaJ and GrpE under control of a strong inducible promoter is described for use in the prevention of inclusion body formation during the manufacture of foreign proteins by expression of the cloned in Escherichia coli or other bacterial hosts. Co-expression of genes for prourokinase and an operon containing the DnaK and DnaJ genes led to most of the prourokinase remaining soluble The prourokinase accumulated in inclusion bodies in cells lacking the artificial operon.

L2 ANSWER 13 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2000:164663 CAPLUS
DOCUMENT NUMBER: 132:190463
TITLE: Preparation of fusion protein containing Escherichia coli thioredoxin derivative using a soluble expression system
INVENTOR(S): Cui, Libin; Ma, Qingjun
PATENT ASSIGNEE(S): Biological Engineering Inst., Academy of Military Medicine, P.L.A., Peop. Rep. China
SOURCE: Faming Zhuanli Shenqing Gongkai Shuomingshu, 14 pp.
CODEN: CNXXEV
DOCUMENT TYPE: Patent
LANGUAGE: Chinese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CN 1189539	A	19980805	CN 1997-100362	19970131 <--

PRIORITY APPLN. INFO.: CN 1997-100362 19970131
AB Described is a soluble expression system for preparation of fusion protein containing thioredoxin and a heterologous protein in prokaryotes. The method can avoid the formation of inclusion bodies and simplify the purification process to obtain biol. active proteins. The method is improved by using a thioredoxin derivative obtained by inserting polymeric His and Gly (e.g. (His)5-Gly) into the active site between 33-Gly and 34-Pro. An enzymic hydrolysis site (e.g. IgA protease-specific cutting site) is also placed between the thioredoxin derivative and the heterologous protein. The soluble expression method was used for expression of human G-CSF in Escherichia coli.

L2 ANSWER 14 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1998:756773 CAPLUS
DOCUMENT NUMBER: 130:76809
TITLE: Over-expression of G-CSF in Escherichia coli and fast purification protocol
AUTHOR(S): Li, Fu-Sheng; Gong, Hui-Yu; Zhao, Bing-Wen; Yu, Cai-Ling; Hou, Bin; Chen, Ai-Jun; Zhang, Zhi-Qing; Hou, Yun-De
CORPORATE SOURCE: State Key Lab. Mol. Virol. Genetic Eng., Inst. Virol. CAPM, Beijing, 100052, Peop. Rep. China
SOURCE: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (1998), 14(5), 479-484
CODEN: ZSHXF2; ISSN: 1007-7626
PUBLISHER: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB Recombinant human granulocyte colony stimulating factor (rhG-CSF) is mainly used in neutropenia induced by cytotoxic chemotherapy in clin. practice. After a Chinese human G-CSF cDNA was cloned, the 5' terminal sequence in G-CSF cDNA was thoroughly modified in order to raise the expression level. Plasmid pBV220/G-CSF/2-174 was constructed by inserting the modified gene into the pBV220 vector. Over 50% of the cellular protein was the rhG-CSF. As G-CSF was in inclusion body in E. coli, a simple and stable purification protocol was established, which was very suitable for large-scale purification Firstly, inclusion body was extracted from E. coli, and then, 8 mol/L urea was used to lysis the inclusion body. G -CSF protein was renatured by dilution And the pure G-CSF was recovered by one-step SP-Sepharose FF chromatog. The relative activity of purified G-CSF reached to 3.4 x 108 U/mg protein. A total G-CSF activity from 1 L fermentation was about 1.06 x 1011 U. As demonstrated by N-terminal amino acid sequencing, methionine had thoroughly been removed, so this kind of purified G-CSF may have a low immunogenicity and toxicity.

L2 ANSWER 15 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2000:720957 CAPLUS
DOCUMENT NUMBER: 135:29588
TITLE: Expression of cDNA for recombinant human granulocyte colony-stimulating factor in Escherichia coli and characterization of the protein
AUTHOR(S): Zhang, Shu; Ye, Qinong
CORPORATE SOURCE: The School of Oncology, Beijing Medical University, Beijing Cancer Hospital, Beijing, 100036, Peop. Rep. China
SOURCE: Chinese Journal of Cancer Research (1998),

10(4), 256-259
CODEN: CJCRFH; ISSN: 1000-9604
PUBLISHER: Chinese Journal of Cancer Research
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Objective: To determine the biol. activity of rhG-CSF and it's characterization. Methods: The prokaryotic expression vector pG01 containing human G-CSF cDNA were constructed with DNA recombination technol. Results: We had achieved high level expression of the human G-CSF in E. coli, where it represented at least 23.6% of the total protein as determined from SDS-PAGE gels. The human G-CSF was expressed as inclusion bodies in E.coli. The inclusion bodies were solubilized in a solution containing 7M urea, renatured by dialysis, isolated and purified by DEAE-sepharose CL-6B ion exchange and Superdex 75 gel filtration chromatog. The purified rhG-CSF was confirmed by coincidence of biol. activity and protein demonstrated by SDS-PAGE. It was homogeneous with respect to mol. Wt (18400). The purity of the rhG-CSF might be >90 per cent. Conclusion: The purified rhG-CSF in our laboratory had dramatically the biol. activity of regulating proliferation and differentiation of the human G-CSF-dependent cell line NSF-1 and the progenitor cells of granulocytes of human bone marrow.
REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 16 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1998:252165 CAPLUS
DOCUMENT NUMBER: 128:317751
ORIGINAL REFERENCE NO.: 128:62857a,62860a
TITLE: Recombinant expression and identification of human granulocyte colony-stimulating factor cDNA in Escherichia coli
AUTHOR(S): Fang, Xiangdong; Ma, Li; Gao, Jimin; Huang, Shuqi; Wang, Xiaoning
CORPORATE SOURCE: Institute Molecular Immunology, First Military Medical Univ., Canton, 510515, Peop. Rep. China
SOURCE: Zhongguo Shenghua Yaowu Zazhi (1998), 19(1), 1-5
CODEN: ZSYZFP; ISSN: 1005-1678
PUBLISHER: Zhongguo Shenghua Yaowu Zazhi Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB The gene for human granulocyte colony-stimulating factor (G-CSF) was amplified by RT-PCR and inserted into the expression vector pJGW1. The recombinant human G-CSF (rhG-CSF) was expressed in E. coli DH5α that contained plasmid pJGW1-hG-CSF and pGP1-2. G-CSF (mol. weight .apprx.19 kilodaltons) accounted for >30% of total protein of the recombinant E. coli. Western-blot revealed that the 19-kilodalton protein shared specific antigenicity with native G-CSF. The rhG-CSF was isolated and purified up to 98% purity by inclusion body isolation, refolding and CM-Sepharose Fast Flow ion exchange chromatog.

L2 ANSWER 17 OF 18 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1993:1980 CAPLUS
DOCUMENT NUMBER: 118:1980
ORIGINAL REFERENCE NO.: 118:435a,438a
TITLE: Improving the resolubilization of proteins synthesized in an heterologous host and accumulated as inclusion bodies
INVENTOR(S): Ambrosius, Dorothea; Dony, Carola; Rudolph, Rainer

PATENT ASSIGNEE(S): Boehringer Mannheim G.m.b.H., Germany
SOURCE: Eur. Pat. Appl., 18 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 500108	A2	19920826	EP 1992-102864	19920220 <--
EP 500108	A3	19930407		
EP 500108	B1	19961016		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, PT, SE				
DE 4105480	A1	19920827	DE 1991-4105480	19910221 <--
AU 9210948	A	19920827	AU 1992-10948	19920214 <--
AU 641081	B2	19930909		
CA 2061569	A1	19920822	CA 1992-2061569	19920220 <--
CA 2061569	C	20001024		
FI 9200742	A	19920822	FI 1992-742	19920220 <--
FI 106029	B1	20001115		
NO 9200671	A	19920824	NO 1992-671	19920220 <--
NO 300329	B1	19970512		
ZA 9201230	A	19921125	ZA 1992-1230	19920220 <--
JP 05244977	A	19930924	JP 1992-33257	19920220 <--
JP 2528232	B2	19960828		
HU 68021	A2	19950404	HU 1992-548	19920220 <--
HU 214881	B	19980728		
IL 101024	A	19960618	IL 1992-101024	19920220 <--
AT 144284	T	19961115	AT 1992-102864	19920220 <--
ES 2093122	T3	19961216	ES 1992-102864	19920220 <--
CZ 282744	B6	19970917	CZ 1992-499	19920220 <--
US 5578710	A	19961126	US 1993-139054	19931021 <--

PRIORITY APPLN. INFO.: DE 1991-4105480 A 19910221
US 1992-837779 B1 19920214

AB The resolubilization of proteins that accumulate as inclusion bodies when synthesized in an heterologous host is made more efficient by synthesizing the protein with an N- or C-terminal addition of a hydrophilic peptide of 5-20 amino acids. The peptide is made up of amino acids with a neg. relative hydrophobicity such as Cys, Ser, Gln, Lys, Arg, or Pro. A series of peptides for addition to the N-terminus of a protein were designed and oligonucleotides encoding them were introduced at the 5'-end of a sequence encoding granulocyte colony-stimulating factor (G-CSF) and the genes expressed in Escherichia coli. Inclusion bodies were prepared, and solubilized in concentrated guanidine. hydrochloride and renatured in an arginine-based buffer by methods of the prior art. Recovery of G-CSF was measured by an in vitro test with a G-CSF-dependent cell line. After optimization of renaturation conditions, recoveries of ≥80% of the biol. activity could be found with longer, more hydrophobic, peptides having greater effects than shorter ones with two adjacent glutamate residues having a significant effect.

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AT 16:33:49 ON 22 DEC 2008
FILE 'MEDLINE' ENTERED AT 16:33:49 ON 22 DEC 2008
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     FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE' ENTERED AT 15:10:58 ON 22 DEC 2008
L1            27 S G-CSF (P)(INCLUSION BODY) AND PD<=20030611
L2            18 DUP REM L1 (9 DUPLICATES REMOVED)
L3             1 S L2 AND NON-DENATUR?

=> S (correct (3A) Fold?) (P) protein (P) (inclusion body) AND pd<=20030611
   1 FILES SEARCHED...
L4           52 (CORRECT (3A) FOLD?) (P) PROTEIN (P) (INCLUSION BODY) AND PD<=20
               030611

=> Dup Rem L4
PROCESSING COMPLETED FOR L4
L5            19 DUP REM L4 (33 DUPLICATES REMOVED)
                 ANSWERS '1-14' FROM FILE MEDLINE
                 ANSWER '15' FROM FILE BIOSIS
                 ANSWERS '16-19' FROM FILE CAPLUS

=> D ibib abs L5 1-19

L5   ANSWER 1 OF 19     MEDLINE on STN                  DUPLICATE 1
ACCESSION NUMBER:   2004161387     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 14983099
TITLE:              pH-responsive polymer-assisted refolding of urea- and
                    organic solvent-denatured alpha-chymotrypsin.
AUTHOR:             Roy I; Gupta M N
CORPORATE SOURCE:   Chemistry Department, Indian Institute of Technology,
                    Delhi, Hauz Khas, New Delhi 110016, India.
SOURCE:             Protein engineering, (2003 Dec) Vol. 16, No. 12,
                    pp. 1153-7.
                    Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY:       England: United Kingdom
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
                    (RESEARCH SUPPORT, NON-U.S. GOV'T)
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LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200410
ENTRY DATE: Entered STN: 2 Apr 2004
Last Updated on STN: 22 Oct 2004
Entered Medline: 21 Oct 2004

AB A pH-responsive polymer Eudragit S-100 has been found to assist in correct folding of alpha-chymotrypsin denatured with 8 M urea and 100 mM dithiothreitol at pH 8.2. The complete activity could be regained within 10 min during refolding. Both native and refolded enzymes showed emission of intrinsic fluorescence with lambda(max) of 342 nm. Gel electrophoresis showed that the presence of Eudragit S-100 led to dissociation of multimers followed by the appearance of a band at the monomer position. The unfolding (by 8 M urea) and folding (assisted by the polymer) also led to complete renaturation of alpha-chymotrypsin initially denatured by 90% dioxane. The implications of the data in recovery of enzyme activity from inclusion bodies and the interesting possibility in the in vivo context of reversing protein aggregation in amyloid-based diseases have been discussed.

L5 ANSWER 2 OF 19 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2003063197 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12573253
TITLE: Optimized overproduction, purification, characterization and high-pressure sensitivity of the prion protein in the native (PrP(C)-like) or amyloid (PrP(Sc)-like) conformation.
AUTHOR: Alvarez-Martinez Maria Teresa; Torrent Joan; Lange Reinhard; Verdier Jean-Michel; Balny Claude; Liautard Jean-Pierre
CORPORATE SOURCE: INSERM U431, CC100, Dept Biologie Sante, Universite de Montpellier 2, Place Eugene Bataillon, F-34095 Montpellier Cedex 5, France.
SOURCE: Biochimica et biophysica acta, (2003 Feb 21) Vol. 1645, No. 2, pp. 228-40.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200305
ENTRY DATE: Entered STN: 8 Feb 2003
Last Updated on STN: 8 May 2003
Entered Medline: 7 May 2003

AB Overproduction and purification of the prion protein is a major concern for biological or biophysical analysis as are the structural specificities of this protein in relation to infectivity. We have developed a method for the effective cloning, overexpression in Escherichia coli and purification to homogeneity of Syrian golden hamster prion protein (SHaPrP(90-231)). A high level of overexpression, resulting in the formation of inclusion bodies, was obtained under the control of the T7-inducible promoter of the pET15b plasmid. The protein required denaturation, reduction and refolding steps to become soluble and attain its native conformation. Purification was carried out by differential centrifugation, gel filtration and reverse phase chromatography. An improved cysteine oxidation protocol using oxidized glutathione under denaturing conditions, resulted in the recovery of a higher yield of chromatographically pure protein. About 10 mg of PrP protein per liter of bacterial culture was obtained. The recombinant protein was identified by monoclonal antibodies and its integrity was confirmed by

electrospray mass spectrometry (ES/MS), whereas correct folding was assessed by circular dichroism (CD) spectroscopy. This protein had the structural characteristics of PrP(C) and could be converted to an amyloid structure sharing biophysical and biochemical properties of the pathologic form (PrP(Sc)). The sensitivity of these two forms to high pressure was investigated. We demonstrate the potential of using pressure as a thermodynamic parameter to rescue trapped aggregated prion conformations into a soluble state, and to explore new conformational coordinates of the prion protein conformational landscape.

L5 ANSWER 3 OF 19 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2003014239 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12007009
TITLE: The effect of N-terminal changes on arginyl-tRNA synthetase from Escherichia coli.
AUTHOR: Liu Wen; Liu Mo-Fang; Xia Xia; Wang En-Duo; Wang Ying-Lai
CORPORATE SOURCE: State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, the Chinese Academy of Sciences, Shanghai 200031, China.. wed@server.shcnc.ac.cn
SOURCE: Sheng wu hua xue yu sheng wu wu li xue bao Acta biochimica et biophysica Sinica, (2002 Mar) Vol. 34, No. 2, pp. 131-7.
Journal code: 20730160R. ISSN: 0582-9879.
PUB. COUNTRY: China
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200307
ENTRY DATE: Entered STN: 11 Jan 2003
Last Updated on STN: 3 Jul 2003
Entered Medline: 2 Jul 2003
AB An Asn(2) deleted mutant of Escherichia coli arginyl-tRNA synthetase deleted Asn(2) and a chimera mutant, in which the N-terminal 23 amino acid residues of yeast arginyl-tRNA synthetase were appended to the N-terminus of Escherichia coli synthetase, were synthesized and studied. The expression of the deletion and chimera mutants in Escherichia coli formed inclusion bodies, presumably due to improper folding of the proteins. Relative to the native enzyme, the deletion mutant showed full amino acid activation activity and a 26% reduction in aminoacylation activity, while the chimera mutant lost 93% and 96% activities in aminoacid activation and aminoacylation, respectively, and did not aminoacylate yeast tRNA(Arg) at all. The mutant deleted Asn(2) and Ile(3) was able to be expressed in Escherichia coli but not stable to be purified. The emission maximum wavelength in the fluorescence spectra of the chimera mutants shifted to longer one and the corresponding intensities decreased, when compared with those of the native enzyme. The data show that the conformation of the mutants are different and the tryptophan residues in the mutants are more exposed than those in the native enzyme. An estimate of the secondary structure of the mutant enzymes from their far ultraviolet CD spectra showed that the chimera mutant contained less alpha-helix, more beta-sheet and slightly higher fraction of random coil, as compared with the native enzyme. The results indicate that an intact N-terminal domain of E.coli arginyl-tRNA synthetase is important to its activity and correct folding.

L5 ANSWER 4 OF 19 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2001061116 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11054122

TITLE: Protein disulfide isomerase-mediated cell-free assembly of recombinant interleukin-12 p40 homodimers.
AUTHOR: Martens E; Alloza I; Scott C J; Billiau A; Vandenbroeck K
CORPORATE SOURCE: Rega Institute for Medical Research, University of Leuven, Belgium.
SOURCE: European journal of biochemistry / FEBS, (2000 Nov) Vol. 267, No. 22, pp. 6679-83.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200012
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 22 Mar 2001
Entered Medline: 28 Dec 2000

AB Interleukin-12 (IL-12) is a heterodimeric cytokine composed of two subunits, p35 and p40. The disulfide-linked homodimer (p40)2 has been shown to be a potent IL-12 antagonist. In the present study, the p40 subunit was refolded from Escherichia coli inclusion bodies. Formation of (p40)2 was greatly increased in a redox buffer containing reduced and oxidized glutathione, but was not significantly affected by the cosolvents urea, GdnHCl or Chaps. While protein disulfide isomerase (PDI), GroEL/ES or DnaK/J/GrpE suppressed aggregation during refolding of p40, only DnaK/J/GrpE and PDI enhanced p40 dimerization. Oxidative assembly of p40 into (p40)2 by PDI, but not suppression of aggregation, was strongly dependent on inclusion of BSA in the refolding buffer. It is concluded that both chaperone-like and disulfide isomerase effects are essential for correct folding of p40 into dimers.

L5 ANSWER 5 OF 19 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2001086677 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10970778
TITLE: Function of the N-terminal propeptide of an aminopeptidase from Vibrio proteolyticus.
AUTHOR: Zhang Z Z; Nirasawa S; Nakajima Y; Yoshida M; Hayashi K
CORPORATE SOURCE: Applied Enzymology Laboratory, National Food Research Institute, Tsukuba, Ibaraki 305-8642, Japan.
SOURCE: The Biochemical journal, (2000 Sep 15) Vol. 350 Pt 3, pp. 671-6.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 22 Mar 2001
Entered Medline: 18 Jan 2001

AB An aminopeptidase from Vibrio proteolyticus was translated as a preproprotein consisting of four domains: a signal peptide, an N-terminal propeptide, a mature region and a C-terminal propeptide. Protein expression and analysis of the activity results demonstrated that the N-terminal propeptide was essential to the formation of the active enzyme in Escherichia coli. Urea dissolution of inclusion bodies and dialysis indicated that the N-terminal propeptide could facilitate the correct folding of the enzyme in vitro. Using L-Leu-p-nitroanilide as the substrate, the kinetic parameters (k(cat) and K(m)) of the pro-aminopeptidase and processed aminopeptidases

were analysed. The results suggested that the N-terminal propeptide inhibited enzyme activity of the mature region. In contrast, the C-terminal propeptide did not show evidence of forming an active enzyme, of correctly folding in vitro or of inhibiting the active region.

L5 ANSWER 6 OF 19 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 1999289510 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10360984
TITLE: Expression of a synthetic gene encoding canine milk lysozyme in Escherichia coli and characterization of the expressed protein.
AUTHOR: Koshiba T; Hayashi T; Miwako I; Kumagai I; Ikura T; Kawano K; Nitta K; Kuwajima K
CORPORATE SOURCE: Division of Biological Sciences, Graduate School of Science, Hokkaido University, Kita-ku, Sapporo 060-0810, Japan.
SOURCE: Protein engineering, (1999 May) Vol. 12, No. 5, pp. 429-35.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 30 Jul 1999
Last Updated on STN: 30 Jul 1999
Entered Medline: 21 Jul 1999
AB A high-expression plasmid of the canine milk lysozyme, which belongs to the family of calcium-binding lysozymes, was constructed in order to study its physico-chemical properties. Because the cDNA sequence of the protein has not yet been determined, a 400 base-pair gene encoding canine milk lysozyme was first designed on the basis of the known amino acid sequence. The gene was constructed by an enzymatic assembly of 21 chemically synthesized oligonucleotides and inserted into an Escherichia coli expression vector by stepwise ligation. The expression plasmid thus constructed was transformed into BL21(DE3)/pLysS cells. The gene product accumulated as inclusion bodies in an insoluble fraction. Recombinant canine milk lysozyme was obtained by purification and refolding of the product and showed the same characteristics in terms of bacteriolytic activity and far- and near-UV circular dichroism spectra as the authentic protein. The NMR spectra of refolded lysozyme were also characteristic of a native globular protein. It was concluded that recombinant canine milk lysozyme was folded into the correct native structure. Moreover, the thermal unfolding profiles of the refolded recombinant lysozyme showed a stable equilibrium intermediate, indicating that the molten globule state of this protein was extraordinarily stable. This expression system of canine milk lysozyme will enable biophysical and structural studies of this protein to be extended.

L5 ANSWER 7 OF 19 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 1999288227 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10336874
TITLE: Folding and purification of a recombinantly expressed interferon regulatory factor, IRF-4.
AUTHOR: Moellering B J; Yoshinaga S K; Hui A; Delaney J M; Hara S; Narhi L O; Westcott K R
CORPORATE SOURCE: Amgen Inc., One Amgen Center Drive, Thousand Oaks, California 91320-1789, USA.
SOURCE: Protein expression and purification, (1999 Jun) Vol. 16, No. 1, pp. 160-70.

Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 27 Jul 1999
Last Updated on STN: 27 Jul 1999
Entered Medline: 12 Jul 1999

AB Interferon regulatory factor 4 (IRF-4), an intracellular, multidomain protein, is a member of the interferon regulatory factor family and a lymphoid-specific transcription factor that can form a ternary complex with DNA and the transcription factor PU.1. Recombinant human IRF-4 was expressed in Escherichia coli and purified from the soluble cell extract and the insoluble inclusion bodies. The inclusion bodies were solubilized with guanidinium-hydrochloride and sequentially buffer exchanged into urea- and then NaCl-containing solutions. This two-step process for the removal of the denaturants was the critical step to allow for the correct folding of IRF-4. Following purification through immobilized metal affinity, hydrophobic interaction, and gel permeation chromatographies, the renatured protein was shown to be structurally and physically equivalent to a sample of IRF-4 produced in the soluble fraction of E. coli cells. This was confirmed by near and far UV circular dichroism analysis, including thermal stability analysis. The purified IRF-4 was also shown to be capable of binding DNA in a PU.1-dependent manner by electrophoretic mobility shift analysis. The protein folding and purification methods are suitable for producing large quantities of full-length IRF-4.

L5 ANSWER 8 OF 19 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 1999081093 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9865495
TITLE: Interferon-gamma is a target for binding and folding by both Escherichia coli chaperone model systems GroEL/GroES and DnaK/DnaJ/GrpE.
AUTHOR: Vandenbroeck K; Billiau A
CORPORATE SOURCE: Laboratory of Immunobiology, Rega Institute for Medical Research, Leuven, Belgium.
SOURCE: Biochimie, (1998 Aug-Sep) Vol. 80, No. 8-9, pp. 729-37. Ref: 49
Journal code: 1264604. ISSN: 0300-9084.
PUB. COUNTRY: France
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 2 Apr 1999
Last Updated on STN: 2 Apr 1999
Entered Medline: 24 Mar 1999

AB IFN-gamma can be physicochemically distinguished from interferons-alpha, -beta or -omega through the loss of its tertiary structure and biological activity upon exposure to acid or heat. This loss is due to the irreversible aggregation of an unfolded or partially folded state. The conformational instability of IFN-gamma is reflected by its impairment to fold properly when overexpressed in Escherichia coli, resulting in its accumulation in cytoplasmic inclusion bodies.
Chaperones were originally identified as a heterogeneous group of proteins that mediate the folding and correct

assembly of various polypeptide substrates, and protect thermolabile proteins against inactivation. In either of both cases, chaperones prevent irreversible misfolding by assisting the substrate protein along its pathway to a stable tertiary conformation. Among the best characterized chaperones are the Escherichia coli Hsp60 and Hsp70 heat shock protein complexes, i.e., GroEL/GroES and DnaK/DnaJ/GrpE. They exhibit entirely different reaction mechanisms, which, however, both depend on hydrolysis of ATP. The unfolding of recombinant IFN-gamma by acid or heat can be used as a tool to assess its in vitro interaction with each of both chaperone systems at physiological temperature (35 degrees C). Using such an experimental set-up, both the DnaK and GroEL chaperone systems appeared to form complexes with IFN-gamma from which correctly folded protein was released in an ATP-dependent manner. In addition to the biotechnological implication of these observations, the relevance to de novo folding of IFN-gamma is discussed.

L5 ANSWER 9 OF 19 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 1998151283 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9492319
TITLE: Large-scale production, purification and refolding of the full-length cellular prion protein from Syrian golden hamster in Escherichia coli using the glutathione S-transferase-fusion system.
AUTHOR: Volkel D; Blankenfeldt W; Schomburg D
CORPORATE SOURCE: Department of Structure Research, GBF-National Research Centre for Biotechnology, Braunschweig, Germany.. dvo@gbf-braunschweig.de
SOURCE: European journal of biochemistry / FEBS, (1998 Jan 15) Vol. 251, No. 1-2, pp. 462-71.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 7 Apr 1998
Last Updated on STN: 7 Apr 1998
Entered Medline: 23 Mar 1998

AB Until quite recently, high-level expression of full-length cellular prion protein (Prp(c)) in bacterial cells was not possible. We describe here the effective purification of mature Syrian golden hamster PrPc (residues 23-231) as a C-terminal fusion to glutathione S-transferase (GST) from inclusion bodies expressed in Escherichia coli. Purification of the denatured fusion protein was simplified greatly by the introduction of a C-terminal histidine anchor, leading to 255 mg pure GST-PrPc-His6/l bacterial broth, which could be refolded easily by dilution in 20 mM Tris, 5 mM dithiothreitol, 1 mM EDTA, pH 9.0. Refolding was monitored by following GST activity. Mature Syrian hamster PrPc (residues 23-231) was released from the refolded fusion protein by thrombin digestion, yielding 73 mg homogeneous protein/l bacterial culture after purification. The recombinant protein was identified by monoclonal antibodies, Edman sequencing and matrix-assisted laser-desorption/ionization MS. Correct folding was confirmed by near-ultraviolet circular dichroism spectroscopy. Samples resulting from different purification steps were sensitive to proteinase K digestion and showed no signs of infectivity in animal experiments, demonstrating that the PrPc produced is identical with the cellular isoform. The presented purification procedure should prove useful for the production of other GST-fusion proteins.

L5 ANSWER 10 OF 19 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 1998216953 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9557874
TITLE: Expression, purification, and biochemical characterization of a recombinant lectin of Sarcocystis muris (Apicomplexa) cyst merozoites.
AUTHOR: Klein H; Loschner B; Zyto N; Portner M; Montag T
CORPORATE SOURCE: Paul-Ehrlich-Institut, Federal Agency for Sera and Vaccines, FG Parasitologie/Diagnostika, Langen, Germany.. HARALD.KLEIN@EM.UNI-FRANKFURT.DE
SOURCE: Glycoconjugate journal, (1998 Feb) Vol. 15, No. 2, pp. 147-53.
Journal code: 8603310. ISSN: 0282-0080.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 25 Jun 1998
Last Updated on STN: 25 Jun 1998
Entered Medline: 15 Jun 1998

AB The mature major microneme protein of Sarcocystis muris cyst merozoites, which is known as a dimeric lectin with high affinity to galactose and some of its derivatives, was expressed in Escherichia colias a histidine-tagged fusion protein. The recombinant polypeptide, which was recognized by a monoclonal antibody directed against the native lectin, was purified from inclusion bodies after solubilization and refolding, using a combination of metal chelate and lactose affinity chromatography. The apparent molecular mass of the refolded polypeptide as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoreses was 16 kDa, whereas gel filtration chromatography clearly demonstrated that the recombinant protein, like its native counterpart, exists as a homodimer of two non-covalently associated subunits. Inhibition of haemagglutination suggests that the combining site of the recombinant lectin recognizes N-acetyl-galactosamine as the dominant sugar, thus confirming the correct folding of the monosaccharide combining site in the renatured lectin. To the best of our knowledge, this work represents the first reported detailed characterization of a recombinant lectin from apicomplexan parasites, and may contribute to a better understanding of the process of host cell recognition and invasion by these obligate intracellular protozoa.

L5 ANSWER 11 OF 19 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 1997083782 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8930126
TITLE: Renaturation of SPARC expressed in Escherichia coli requires isomerization of disulfide bonds for recovery of biological activity.
AUTHOR: Bassuk J A; Braun L P; Motamed K; Baneyx F; Sage E H
CORPORATE SOURCE: Department of Biological Structure, University of Washington, Seattle 98195, USA.
CONTRACT NUMBER: GM-40711 (United States NIGMS)
HL-18645 (United States NHLBI)
P50-DK-47659 (United States NIDDK)
SOURCE: The international journal of biochemistry & cell biology, (1996 Sep) Vol. 28, No. 9, pp. 1031-43.
Journal code: 9508482. ISSN: 1357-2725.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.)

(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 28 Jan 1997
Last Updated on STN: 6 Feb 1998
Entered Medline: 7 Jan 1997

AB SPARC (secreted protein acidic and rich in cysteine, also known as osteonectin and BM-40) belongs to a group of secreted macromolecules that modulate cellular interactions with the extracellular matrix. During vertebrate embryogenesis, as well as in tissues undergoing remodeling and repair, the expression pattern of SPARC is consistent with a fundamental role for this protein in tissue morphogenesis and cellular differentiation. Human SPARC was cloned by the polymerase chain reaction from an endothelial cell cDNA library and was expressed in Escherichia coli as a biologically active protein. Two forms of recombinant SPARC (rSPARC) were recovered from BL21(DE3) cells after transformation with the plasmid pSPARCwt: a soluble, monomeric form that is biologically active (Bassuk et al., 1996, Archiv. Biochem. Biophys. 325, 8-19), and an insoluble form sequestered in inclusion bodies. Aggregated rSPARC was unfolded by urea treatment, purified by nickel-chelate affinity chromatography, and renatured by gradual removal of the denaturant. Proper isomerization of the disulfide bonds was achieved in the presence of a glutathione redox couple. After final purification by high resolution gel filtration chromatography, a monomeric form of rSPARC displaying biological activity was obtained. The recombinant protein inhibited the spreading and synthesis of DNA by endothelial cells, two properties characteristic of the native protein. We conclude that the information for the correct folding of rSPARC resides in the primary structure of the protein, and suggest that post-translational modifications are required neither for folding nor for biological activity.

L5 ANSWER 12 OF 19 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 1996428683 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8831785
TITLE: Probing the structural role of an alpha beta loop of maltose-binding protein by mutagenesis: heat-shock induction by loop variants of the maltose-binding protein that form periplasmic inclusion bodies.
AUTHOR: Betton J M; Boscus D; Missiakas D; Raina S; Hofnung M
CORPORATE SOURCE: Department des Biotechnologies, Institut Pasteur, Paris, France.
SOURCE: Journal of molecular biology, (1996 Sep 20) Vol. 262, No. 2, pp. 140-50.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19 Dec 1996
Last Updated on STN: 18 Dec 2002
Entered Medline: 7 Nov 1996

AB The maltose-binding protein (MBP) of Escherichia coli is the periplasmic receptor of the maltose transport system. Previous studies have identified amino acid substitutions in an alpha/beta loop of the structure of MBP that are critical for the in vivo folding. To probe genetically the structural role of this surface loop, we generated a library in which the corresponding codons 32 and 33 of malE were mutagenized. The maltose phenotype, which correlates with a biologically active structure of MBP in the periplasm, indicated a considerable

variability in the loop residues compatible with a correct in vivo folding pathway of the protein. By the same genetic screens, we characterized loop-variant MBPs associated with a defective periplasmic folding pathway and aggregated into inclusion bodies. Heat-shock induction with production of misfolded loop variants was examined using both lon-lacZ and htrA-lacZ fusions. We found that the extent of formation of inclusion bodies in the periplasm of E. coli, from misfolded loop variant MBPs, correlated with the level of heat-shock response regulated by the alternate heat-shock sigma factor, sigma 24.

L5 ANSWER 13 OF 19 MEDLINE on STN DUPLICATE 14
ACCESSION NUMBER: 1993072879 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1332541
TITLE: A method for increasing the yield of properly folded recombinant fusion proteins: single-chain immunotoxins from renaturation of bacterial inclusion bodies.
AUTHOR: Buchner J; Pastan I; Brinkmann U
CORPORATE SOURCE: Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.
SOURCE: Analytical biochemistry, (1992 Sep) Vol. 205, No. 2, pp. 263-70.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 22 Jan 1993
Last Updated on STN: 20 Apr 2002
Entered Medline: 1 Dec 1992
AB Many proteins produced in Escherichia coli accumulate in inclusion bodies. We have systematically evaluated the parameters that affect the refolding and renaturation of enzymatically active molecules from bacterial inclusion bodies containing a recombinant single-chain immunotoxin, B3(Fv)-PE38KDEL. This recombinant molecule is composed of the variable domains of monoclonal antibody B3 (B3(Fv)) fused to a truncated mutant form of Pseudomonas exotoxin A (PE38KDEL). This immunotoxin kills carcinoma cells in vitro, causes tumor regression in animal tumor models, and is being developed as an anti-cancer therapeutic agent (Brinkmann et al., 1991, Proc. Natl. Acad. Sci. USA 88, 8616-8620). Like many other recombinant proteins, B3(Fv)-PE38KDEL is produced in E. coli in inclusion bodies and must be denatured and refolded to become active. This requires correct folding, formation of native disulfide bonds, and the association of different domains. All these steps are strongly dependent on the renaturation conditions used. Optimum conditions of refolding were obtained by the addition of reduced and oxidized thiol reagents to promote disulfide bond formation and the addition of a labilizing agent such as L-arginine. Furthermore, the necessity to reactivate proteins at low protein concentrations due to its tendency to aggregate at high concentrations was overcome by a step-by-step addition of denatured and reduced protein into the refolding solution. This approach should be useful for the production of active forms of other recombinant proteins.

L5 ANSWER 14 OF 19 MEDLINE on STN
ACCESSION NUMBER: 1991069858 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1366701
TITLE: Folding of eukaryotic proteins produced in Escherichia coli.

AUTHOR: Kelley R F; Winkler M E
CORPORATE SOURCE: Department of Biomolecular Chemistry, Genentech, Inc., South San Francisco, CA 94080.
SOURCE: Genetic engineering, (1990) Vol. 12, pp. 1-19.
Ref: 48
Journal code: 7907340. ISSN: 0196-3716.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199101
ENTRY DATE: Entered STN: 9 Aug 1995
Last Updated on STN: 9 Aug 1995
Entered Medline: 24 Jan 1991

AB Although intracellular expression in E. coli may result in accumulation of the eukaryotic protein in inclusion bodies, the protein may often be recovered by first solubilizing with denaturant followed by refolding. Some general guidelines for developing a refolding procedure are apparent but the specific protocol must be empirically determined for each protein. Convenient and rapid assays for detecting native protein are critical for developing a refolding procedure. Maintaining solubility during refolding is a common feature of recovery processes. Proper folding should be assessed by a number of methods including activity, spectroscopic and stability measurements. For some proteins, properly folded protein may be obtained by secretion from E. coli; however, secretion does not ensure correct folding and protection from proteolytic degradation.

L5 ANSWER 15 OF 19 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 13
ACCESSION NUMBER: 1996:75360 BIOSIS
DOCUMENT NUMBER: PREV199698647495
TITLE: Improved method for pro-urokinase refolding with inclusion body from recombinant Escherichia coli.
AUTHOR(S): Kubo, Motoki [Reprint author]; Nishi, Akihiro
CORPORATE SOURCE: Dep. Chem. Biochem., Numazu Coll. Technol., 2700 Ooka, Numazu-shi, Shizuoka-ken 410, Japan
SOURCE: Journal of Fermentation and Bioengineering, (1995) Vol. 80, No. 6, pp. 622-624.
CODEN: JFBIEX. ISSN: 0922-338X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Feb 1996
Last Updated on STN: 28 Feb 1996

AB We developed an efficient inclusion body pro-urokinase refolding method from recombinant Escherichia coli. The protein was efficiently refolded when a heat treatment was applied to a protein denaturing solution containing guanidine hydrochloride. The total enzyme activity and the specific activity in response to the 50 degree C heat treatment compared to normal method (25 degree C) were enhanced about 10 and 25%, respectively. Moreover, enhanced protein refolding was also observed in the case of a reduced protein concentration in the protein refolding solution. The result indicates that correct protein folding is closely related to the protein concentration in the refolding solution.

L5 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2000:259841 CAPLUS
DOCUMENT NUMBER: 132:278249

TITLE: Efficient recovery of biologically active β subunit of human nerve growth factor from bacterial inclusion bodies
INVENTOR(S): Rattenholl, Anke; Grossmann, Adelbert; Schwarz, Elisabeth; Rudolph, Rainer
PATENT ASSIGNEE(S): SCIL Proteins GmbH, Germany
SOURCE: Eur. Pat. Appl., 25 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 994188	A1	20000419	EP 1998-119077	19981009 <--
EP 994188	B1	20040107		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AT 257514	T	20040115	AT 1998-119077	19981009
ES 2213861	T3	20040901	ES 1998-119077	19981009
CA 2346257	A1	20000420	CA 1999-2346257	19991011 <--
CA 2346257	C	20050301		
WO 2000022119	A1	20000420	WO 1999-EP7613	19991011 <--
W: AU, BR, CA, JP, KR, US, ZA				
BR 9914393	A	20010626	BR 1999-14393	19991011 <--
JP 2002527062	T	20020827	JP 2000-576009	19991011 <--
AU 765810	B2	20031002	AU 2000-10348	19991011
ZA 2001002780	A	20020704	ZA 2001-2780	20010404 <--

PRIORITY APPLN. INFO.: EP 1998-119077 A 19981009
WO 1999-EP7613 W 19991011

AB A method of preparing biol. active human nerve growth factor β-subunit (NGFβ) from inclusion bodies of the prepro- form is described. The method involves denaturing solubilization of the prepro-form followed by renaturation. The propeptide appears to play an important role in the correct folding of the protein into its biol. active form. Manufacture of the protein in Escherichia coli using a T7 expression system to manufacture the protein as inclusion bodies is demonstrated. Optimization expts. to maximize the recovery of biol. active protein from inclusion bodies solubilized with guanidinium salts are reported. The refolded precursor was assayed for biol. activity in a dorsal root ganglion assay and found to have a biol. activity about half of that of the mature NGFβ. Limited proteolysis with trypsin increased the biol. activity of the precursor.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 17 OF 19 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1999:640887 CAPLUS
DOCUMENT NUMBER: 131:267960
TITLE: Manufacture of proteins as fusion products with peptides that promoter protein folding and the manufacture of correctly-folded proteins
INVENTOR(S): Gan, Zhongru
PATENT ASSIGNEE(S): Tonghua Gantech Biotechnology Ltd., Peop. Rep. China
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9950302	A1	19991007	WO 1998-CN52	19980331 <--
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2324513	A1	19991007	CA 1998-2324513	19980331 <--
AU 9867164	A	19991018	AU 1998-67164	19980331 <--
AU 765574	B2	20030925		
BR 9815788	A	20001128	BR 1998-15788	19980331 <--
EP 1066328	A1	20010110	EP 1998-912192	19980331 <--
EP 1066328	B1	20080917		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2004505601	T	20040226	JP 2000-541204	19980331
RU 2238951	C2	20041027	RU 2000-127730	19980331
CN 1197876	C	20050420	CN 1998-813941	19980331
US 6924120	B1	20050802	US 2000-423100	19980331
AT 408631	T	20081015	AT 1998-912192	19980331
MX 2000PA09564	A	20040310	MX 2000-PA9564	20000929
US 20020164712	A1	20021107	US 2002-54873	20020122 <--
PRIORITY APPLN. INFO.:			US 2000-423100 A1	19980331
			WO 1998-CN52 A	19980331

AB A method of manufacturing a protein that increases the yield of correctly folded proteins by synthesizing it as a fusion protein with a peptide that promotes correct folding is described. The protein may be a chaperonin or a propeptide, e.g. from human growth hormone, that also helps to promote correct folding. The protein manufactured by an expression host is denatured with a chaotropic agent and allowed to renature with the chaperonin helping to direct correct folding. The chaperonin moiety can be removed by chemical cleavage. The method is particularly intended for use in the manufacture of insulin. An assay for screening an amino acid sequence for the ability to improve folding of an insulin precursor using a chimeric protein containing an IMC like sequence linked to an insulin precursor is also described. Use of the human growth hormone propeptide to manufacture a human mini-proinsulin in Escherichia coli is demonstrated. Inclusion bodies containing the protein were solubilized with alkaline urea and renatured with a yield of .apprx.70%. The refolded material was purified by ultrafiltration with a yield of 85%. The insulin was released from the fusion protein with trypsin and the C-terminal arginine of the B chain was removed with carboxypeptidase. Crystalline insulin obtained from this stage had a purity of >99%.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 18 OF 19 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2000:44626 CAPLUS

DOCUMENT NUMBER: 133:2153

TITLE: Inhibition of aggregation side reactions during in vitro protein folding

AUTHOR(S): De Bernardez Clark, Eliana; Schwarz, Elisabeth; Rudolph, Rainer

CORPORATE SOURCE: Department of Chemical Engineering, Tufts University, Medford, MA, 02155, USA

SOURCE: Methods in Enzymology (1999), 309(Amyloid, Prions, and Other Protein Aggregates), 217-236
CODEN: MENZAU; ISSN: 0076-6879
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Overexpressed, recombinant proteins are often sequestered in the form of insol., inactive inclusion bodies. Active proteins can be recovered from the inclusion bodies by solubilization of chaotropic buffer systems and subsequent in vitro folding. However, unproductive side reactions (predominantly aggregation) often compete with correct folding during in vitro folding. Various techniques are described to inhibit aggregation side reactions and to ensure efficient in vitro protein folding. (c) 1999 Academic Press.
REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 19 OF 19 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1994:480725 CAPLUS
DOCUMENT NUMBER: 121:80725
ORIGINAL REFERENCE NO.: 121:14495a,14498a
TITLE: Refolding of recombinant IL-2 in vitro
AUTHOR(S): Xu, Ming-bo; Meng, Wen-hua; Ma, Xian-kai
CORPORATE SOURCE: Inst. Basic Med. Sci., Beijing, 100850, Peop. Rep. China
SOURCE: Shengwu Huaxue Zazhi (1994), 10(3), 376-81
CODEN: SHZAE4; ISSN: 1000-8543
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB Recombinant proteins extracted from inclusion body remain in denaturation status. Refolding (or renaturation) in vitro after in initial purification is a key step in down-stream processing. The fluorescence value decreased slowly during the refolding process of IL-2 and emission fluorescence peak shifted from 316 nm to 348 nm. Similar result was obtained with GM-CSF using the exposition of Trp residue as an indication of protein folding status. Gel filtration HPLC can be used to detect the oligomer of the product during refolding, while reversed phased HPLC can be used to sep. the three different isomers of IL-2 caused by different disulfide formation. From the separation result, the correct folding ratio of IL-2 mols. can be calculated A common method for refolding of recombinant proteins is the dilution method. With this method, the correct folding ratio will decrease when the refolding protein concentration increases. For example, the correct folding ratio was only 30% when IL-2 concentration reached 1mg/mL. If refolding was carried out under very low protein concentration, the large sample volume will be difficult to handle.

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STN INTERNATIONAL SESSION SUSPENDED AT 16:38:39 ON 22 DEC 2008